# BIOSOCIAL INFLUENCES

# ON TODDLER GENDER-LINKED BEHAVIOR

A Thesis

by

JANET SAENZ

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2010

Major Subject: Psychology

Biosocial Influences

on Toddler Gender-Linked Behavior

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Approved by:

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# ABSTRACT


Biosocial Influences

on Toddler Gender-Linked Behavior. (December 2010)

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Chair of Advisory Committee: Dr. Gerianne M. Alexander

There is increasing evidence that biological factors (i.e., hormones) support the development of sex differences in behavior by organizing brain systems in prenatal life. However, the behavioral significance of the surge in reproductive steroids that results from the transient activation of the hypothalamic-pituitary-gonadal (HPG) axis around 3-months of age is largely unknown. The goal of the present study was to investigate the role of early postnatal activation of the HPG axis in the development of sex differences in human behavior.

Participants included 54 children between 18 and 24 months (32 males and 22 females) and their parents. Infants and their caregivers participated in two, eight minute play sessions that were video-taped and later coded for children's aggressive behavior and vocal ability. During each session, children wore an actigraph (Actiwatch, Philips Respironics) to provide a nonbiased assessment of activity levels. In addition, toddler's temperament was measured using the Brief Infant Toddler Social-Emotional Assessment (BITSEA). Saliva samples from each infant were collected at 3-4 months of age and levels of testosterone were measured. Digit ratio levels were also measured at 3-4 months and used as a marker of prenatal androgen levels.

The data indicated that boys were more aggressive, engaged in higher levels or activity, and showed less developed language ability. In addition, our results demonstrated that hormone markers associated with higher (i.e., more male-typical) testosterone were related to more aggressive behaviors, higher activity levels, expression of fewer total words, and a shorter duration of time spent vocalizing. A novel finding was that higher testosterone (i.e., more male-typical) levels in early postnatal life predicted less time spent vocalizing, for both sexes together and within males. The present research suggests that hormone levels in early postnatal life may contribute to the development of gender phenotypes, potentially making this a critical period for the development of language and other gender-linked behaviors.

# DEDICATION

To Annabel, Andrew, and Jayden. I hope each of you is able to achieve your greatest dreams. And remember, there are no limits in life but those you choose for yourself!

# ACKNOWLEDGEMENTS

First, I would like to thank my committee chair, Dr. Alexander, for making graduate school a very rewarding experience. I greatly appreciate all of her time and feedback. Furthermore, I would like to thank my committee members, Dr. Heffer and Dr. Blake, for their guidance and support throughout the course of this research.

I would also like to thank my previous mentors, academic advisors, and friends who have been influential in shaping my career goals and fostering my academic development. I would especially like to thank Susan Pearman, who kindly opened her heart and steered me through the process of higher education, from helping me select adequate attire for my first professional conference to teaching me valuable interviewing skills that I've used time and time again.

In addition, I would like to thank my mother, Esperanza Castellanos, and father, Javier Saenz, for their unconditional love and continuous encouragement. Thanks to my grandparents, aunts, uncles, cousins, brother, sister, and in-laws, for showing me how beautiful a large support system can be. Lastly, I want to thank my husband, Tony Toribio, for all of his patience and love!

# NOMENCLATURE

| | |
|---|---|
| CAH | Congenital Adrenal Hyperplasia |
| HPG | Hypothalamic-Pituitary-Gonadal |
| 2D:4D | Second and Fourth Digits |

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# 1. INTRODUCTION

There is increasing evidence that biological factors (i.e., hormones) support the development of sex differences in behavior by organizing brain systems in prenatal life and influencing responsiveness to gender-linked stimuli following puberty (Breedlove, Cooke, & Jordan, 1999; Hines, 2002; Phoenix, Goy, Gerral, & Young, 1959). However, the behavioral significance of the surge in reproductive steroids that results from the transient activation of the hypothalamic-pituitary-gonadal (HPG) axis around 3-months of age (Chellakootym et al., 2003; Forest, Sizonenko, Cathiard, & Bertrand, 1974; Huhtaniemi, Dunkel, & Perheentupa, 1986) is largely unknown. We argue that this hormonal rush is another critical period for the organization of later behavior. The goal of this research is to investigate the role of early postnatal activation of the HPG axis in the development of sex differences in human behavior.

In other species, higher levels of androgens during critical periods of prenatal life masculinize and defeminize behaviors, such as play, aggression, and sexual behavior (Breedlove et al., 1999). In humans, obvious ethical constraints preclude experimental manipulation of prenatal hormone levels. Instead, behavioral outcomes have been associated with prenatal androgen levels assessed using indirect techniques such as, collecting hormonal concentrations in maternal fluids, comparing healthy children to those born with endocrine disorders, and measuring digit ratios (Cohen-Bendahan, Beek, & Berenbaum, 2005).

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This thesis follows the style of *Developmental Psychology*.

## 1.1 Prenatal Hormones

### *1.1.1 Maternal fluids*

Obtaining maternal fluids is an invasive procedure that is becoming less common in studies of human development. Hines and colleagues (2002) used blood samples of pregnant mothers and found a positive relationship between a mother's testosterone level and the masculine-typical gender role behavior of their daughters when the girls were three and a half years old. No associations were found between maternal hormone levels and gender role behavior for boys.

### *1.1.2 Endocrine disorders*

Another way to study the effects of elevated levels of androgen exposure is to compare individuals with Congenital Adrenal Hyperplasia (CAH) to normal controls. CAH is a genetic disorder which causes prenatal hormonal abnormality; decreased cortisol production and increased adrenal androgens, such as testosterone. As a result of having high prenatal testosterone levels, these girls are usually born with ambiguous genitalia (Wudy, Dorr, Solleder, Djalali, & Homoki, 1999). In addition, girls with CAH have been shown to be more energetic (Berenbaum, Duck, & Bryk, 2000; Berenbaum & Resnick, 1997), play more with boy peers and boy toys (Berenbaum & Hines, 1992), and are more likely to label themselves and be labeled by others as tomboys (Ehrhardt & Meyer-Bahlburg, 1981).

### *1.1.3 Digit ratios*

Digit ratios is a particularly useful method for obtaining prenatal androgen levels in postnatal life because it is cost effective, time efficient, and non-invasive, therefore enabling its use across various age groups. Digit ratios are obtained by measuring the length of the second and fourth digits (2D:4D) and are thought to be a proxy measure for androgen levels, such that smaller ratios are associated with higher levels of prenatal androgens (Lutchmaya, Baron-Cohen, Raggatt, Knickmeyer, & Manning, 2004; Manning, Bundred, Newton, & Flanagan, 2003). Because digit ratios is a proxy measure, its use is controversial (Putz, Gaulin, Sporter, & McBurney, 2004). However, the association between digit ratios and prenatal androgens is supported by studies showing that the 2D:4D ratio tends to be smaller in males compared to females (Manning, Scott, Wilson, & Lewis-Jones, 1998) and is smaller in children who have been exposed to higher androgen levels prenatally compared to children who have been exposed to normal levels (Brown, Hines, Fane, & Breedlove, 2002; Okten, Kalyoncu, & Yaris, 2002). Research suggests that a positive correlation among digit ratios and higher levels of prenatal estrogen may also exist (Williams, Greenhalgh, & Manning, 2003).

In males, digit ratios are positively correlated with birth weight and head circumference (Ronalds, Phillips, Godfrey, & Manning, 2002). 2D:4D ratios have also been correlated with numerous behaviors, such as left hand preference (Manning, Trivers, Thornhill, & Singh, 2000) and visuo-spatial perception (Anders & Hampson, 2005). In addition, lower 2D:4D ratios have been correlated with some disorders that are more prevalent in boys, such as Autism Spectrum Disorder and Asperger's Syndrom (Manning, Baron-Cohen, Wheelwright, & Sanders, 2001). Lower 2D:4D ratios have also been associated with childhood behaviors that are thought to be related to prenatal testosterone exposure, such as hyperactivity and poor social cognition (Williams et al., 2003).

These associations between digit ratios and features of psychopathology make it likely that digit ratios may also be related to personality features. In fact, low 2D:4D ratios (male-typical) in women are related to high scores on sensation-seeking, disinhibition, and psychoticism, while high 2D:4D ratios (female-typical) in women are related to neuroticism (Austin, Manning, McInroy, & Mathews, 2002). For males and females, right hand digit ratios are positively correlated with neuroticism and are negatively correlated with agreeableness, for females only (Fink, Manning, & Neave, 2004). A positive association between right hand digit ratios and scores on the agreeableness and conscientiousness factors for both genders, has also been reported (Luxen & Buunk, 2005).

Since digit ratios are associated with features of an individual's personality, we can expect that they would also be associated with aggression, which is one aspect of personality. High relational aggression scores have been found to correlate with low (masculinized), right hand digit ratios, in females only (Benderlioglu & Nelson, 2004). While, high trait physical aggression scores have been found to correlate with low (masculinized) digit ratios, in males only (Bailey & Hurd, 2005). However, despite these interesting associations between digit ratios and aggression in adults, there are currently no studies examining this association in children.

## 1.2 Children's Play

### *1.2.1 Importance of play*

Children use a different mode of communication than most adults. While adults rely on words to voice their ideas, attitudes, and concerns, children convey their understanding of the world through the use of creative play (Porter, Hernandez-Reif, & Jessee, 2009). Play also enables them to express their imagination, aspirations, and fears, compensating for their limited use of language and higher cognitive faculties. Play not only serves as a means of learning and communicating, but also facilitates the course of healing. For this reason, play therapy is one of the most common interventions employed with young children. Since play occurs naturally, its setting has also been used in many research studies that are interested in gaining knowledge on children's behavior and development.

### *1.2.2 Sex differences*

Previous studies have shown that before the age of two children engage in gender-typed play, girls preferring dolls and boys preferring trucks (Caldera, Huston, & O'Brien, 1989; Fagot, 1974). Several theoretical perspectives provide explanations for the development of gender-typed play: the cognitive perspective includes gender schema theory and concepts of gender consistency (Bem, 1981; Martin & Halverson, 1981); the social learning perspective includes modeling and reinforcement of behaviors and attitudes (Bussey & Bandura, 1999); and the biological perspective includes hormonal theories (Collaer & Hines, 1995).

Gender labeling refers to a child's ability to accurately identify themselves and others into male and female gender categories. Providing proper gender labels implies a child's awareness of gender schemas, which may facilitate gender stereotyping. On average children begin using gender labels around 19 months of age, with girls labeling significantly earlier than boys. Gender labeling has been shown to predict increases in gender-typed play. We also know that children engage in less gender-typed play when playing with their mothers than when playing alone, contradicting the idea that parents use differential socialization practices to raise girls and boys. (Zosuls et al., 2009)

Differential socialization states that boys and girls are treated differently as they grow; boys get reinforced for male typical behavior, such as rough and tumble play, while girls get reinforced for female typical behavior, such as playing dress up and house. However, the effect size for most socialization categories is not significant and decreases with the child's age (Lytton & Romney, 1991). Not surprisingly, fathers show greater differential socialization of daughters and sons, than mothers (Lytton & Romney, 1991).

Other studies on the role of biological factors have used children with Congenital Adrenal Hyperplasia (CAH) to test the effects of prenatal elevated levels of testosterone on gender-typed play. In general, boys engage in more rough-and-tumble play than girls, while boys with CAH engage in less rough-and-tumble play than boys without CAH and girls with CAH prefer more masculine playmates than girls without CAH (Hines & Kaufman, 1994). We also know that girls with CAH have more male-typical toy preferences than girls without CAH, while boys with and without CAH do not differ in their toy preferences (Berenbaum & Hines, 1992; Pasterski et al., 2005). Even though mothers and fathers encourage sex-typical play in children with and without CAH, parents give girls with CAH more positive feedback for playing with girl toys than they give girls without CAH (Pasterski et al., 2005). However, this extra reinforcement isn't enough to counterbalance their preferences for male-typical toys.

Additional support for a role of biological factors in the development of gender differences in play comes from findings that similar sex differences in toy preferences have been documented in non-human primates. For example, male vervet monkeys spend more contact time with male preferred toys (e.g., truck, ball) than female vervet monkeys; while female vervet monkeys spend more contact time with female preferred toys (e.g., doll, pot) than male vevet monkeys (Alexander & Hines, 2002). In addition, male rhesus monkeys show less interest in plush toys than female rhesus monkeys, while no gender differences are found for wheeled toys (Hassett, Siebert, & Wallen, 2008). These results are consistent with children's play patterns, indicating that boys have more rigid interests in boy toys, presumably because the structure of these toys supports their higher activity levels, while girls show greater interests in both boy and girl toys. These findings suggest that our sex-typed preferences may be a consequence of hormonal influences and evolutionary propensities.

### 1.3 Aggressive Behavior

In most mammals, including humans, males are more aggressive than females throughout the lifespan. Like other gender-linked behaviors, aggression is thought to be an outcome of complex social and biological influences. Different taxonomies of aggressive behavior exist. Frequently, distinctions are made between physical aggression and verbal aggression, relational aggression and overt aggression, proactive aggression and reactive aggression, and instrumental aggression and hostile aggression.

*1.3.1 Form and function of aggression*

As the body of research in this area increases, a consensus on the conceptualization of this construct has become vital. Researchers now regularly differentiate between the form and function of aggression (Little, Jones, Henrich, & Hawley, 2003). The form of aggression refers to the type of expression, while the function refers to the motive. This division results in four dimensions of aggression: the form is either overt/physical/direct or social/relational/indirect, and the function is either reactive/hostile or proactive/instrumental. Several terms represent the same dimension because these terms are considered to share more similarities with each other than differences (Archer & Coyne, 2005).

Although relational aggression is considered to have greater adaptations than overt aggression, both forms have been associated with maladjustment in children and adolescents; overt aggression with externalizing disorders and relational aggression with internalizing disorders (Card, Stucky, Sawalani, & Little, 2008). The frustration-aggression model explains reactive aggression to be an angry response to perceived threat, frustration, or provocation (Berkowit, 1989). In contrast, social learning theory explains proactive aggression to be a deliberate coercive behavior used as a means of obtaining a desired goal (Bandura, 1978). Even though, both functions of aggression are also associated with maladjustment, reactive aggression is associated with worse psychosocial adjustments in children and adolescents than proactive aggression (Card & Little, 2006).

We know that aggressive toddlers typically engage in overt forms of aggression, rather than relational forms. Unfortunately, the function of a toddler's behavior is more difficult to determine because they posses little speech. For this reason, some researchers have begun to measure "conflict" between or among children (Hay, Castle, & Davies, 2000). There are several ways to measure a child's function of aggression or conflict level. The most common include questionnaires (obtained from parents, peers, or target children themselves) and behavioral observations. Both techniques have advantages and disadvantages.

### *1.3.2 Developmental trajectories*

It's possible to distinguish different types of social-emotional behaviors before the age of two. In fact, while many children express problem behaviors occasionally, less than 5% of boys and girls express inattention, physical aggression toward peers, anxiety, shyness, and sadness frequently; 10 to 16% of boys and girls express behaviors of opposition-defiance and separation distress on a frequent basis and; more than 20% of boys and girls express behaviors of hyperactivity on a frequent basis. Gender differences in areas of inattention, hyperactivity, and physical aggression towards peers are present by 17 months, showing higher incidents of these behaviors in boys compared to girls, and increase by 29 months. We also know that there is stability of interindividual differences among individuals who express problem behaviors before the age of two and after the age of two. (Baillargeon et al., 2007)

In fact, dimensions of physical aggression (e.g., object related aggression, bodily aggression) at age 2 predict dimensions of physical aggression at age 5. In addition, the disposition to engage in physical aggression is highly stable in boys, while less stable in girls. Generally, the frequency of aggression decreases from age 2 to age 5, for both boys and girls. (Cummings, Iannotti, & Zahn-Waxler, 1989)

Specifically, most children decline in the use of physical aggression from the age of 2 to 6, or follow a low level trajectory, with the exception of few (about 14.6%) who follow a stable high level trajectory. Moreover, 67% of children follow low level trajectories for indirect aggression, while 32.1 % follow high rising trajectories. Similar numbers for the use of indirect aggression have been reported among children ages 2-10, with 65% following low level trajectories and 35% following high rising trajectories. (Cote, Vaillancourt, Barker, Nagin, & Tremblay, 2007; Vaillancourt, Miller, Fagbemi, Cote, & Tremblay, 2007)

When physical and indirect aggression are examined together, 62.1% of children seem to follow a pattern of desisting physical aggression and low levels of indirect aggression, 14.2% follow a pattern of desisting physical aggression and rising indirect aggression, and 13.5% follow high level trajectories for both forms of aggression. Girls are more likely than boys to follow trajectories of lowering physical aggression and rising indirect aggression. (Cote et al., 2007)

Childhood aggression directly predicts self-reported violence in males and females, and parents' self-reports of using violence with their own children. For mothers, education and the absence of the child's father also predict violence towards their children. This finding supports the idea that aggression is continuous and stable throughout development. (Temcheff et al., 2008)

### *1.3.3 Sex differences*

Preschool girls display greater relational aggression than preschool boys, while boys display greater physical and verbal aggression than girls (Cote et al., 2007; Ostrov & Keating, 2004). Interestingly, children also receive more relational aggression from female peers and more physical and verbal aggression from male peers. We also know that boys and girls react with different types of aggression to different emotional contexts (Tallandini, 2004).

Although boys generally receive higher scores than girls for direct and indirect aggression, groups of highly aggressive 10-14 year olds tend to consist mostly of girls who primarily engage in indirect aggression (Salmivalli & Kaukiainen, 2004). Adolescent girls tend to exhibit greater forms of indirect aggression, while boys tend to exhibit greater forms of direct aggression (Archer, 2004; Lagerspetz, Bjorkqvist, & Peltonen, 1988). Twelve year old girls and 15-year-old girls exhibit more indirect aggression than 8-year old girls, implying that as girls get older they utilize indirect measures of aggression more frequently (Bjorkqvist, Lagerspetz, & Kaukiainen, 1992).

Various meta-analytical studies examining gender differences in aggression have also been conducted. Early narrative reviews that summarized studies of gender differences in aggression conclusively agreed that males are more aggressive than females (Frodi, Macauley, & Thome, 1977; Maccoby & Jacklin, 1974). However, the first meta-analysis with studies of children under the age of 6, concluded that there was no sex differences in aggression at these early ages of development (Tieger, 1980). In response to this claim, a more extensive analysis was conducted, which concluded that sex differences in aggression before the age of 6 do exist, with boys being more aggressive than girls and being more aggressive towards male playmates than female partners (Maccoby & Jacklin, 1980).

In 1984, the magnitude of gender differences in aggression was said to be small, with a trend showing gender differences to be smaller in more recent studies and larger among studies whose participants' mean age was 6 or younger (Hyde). This same dataset was later reanalyzed by a group of researchers who found that sex differences in aggression are actually not diminishing over time (Knight, Fabes, & Higgins, 1996). It was argued that changes in methodology of the original studies were not being accounted for, and when these changes were controlled for the results showed that gender differences in aggression do exist and are fairly stable across time. A more recent meta-analysis supports these findings by concluding that boys engage in more direct aggression than girls, while girls engage in more indirect aggression than boys (Card et al., 2008). The selected meta-analytical studies reflect a major debate that has existed in this field.

*1.3.4 Factors contributing to aggression*

Other factors that contribute to the development of aggression include: family variables, peer groups, culture, and language development. For example, family risk (e.g., maternal depression, family functioning, family status, socioeconomic class, and a mother's age when her child was born) moderates the association between type of child care and aggression, such that maternal care is associated with lower physical aggression among children from low risk family backgrounds but not among children from high risk family backgrounds (Cote, Borge, Geoffroy, Rutter, & Tremblay, 2009). We also know that family risk and the sex of the child moderate the association between emotional problems and child care, such that maternal care is associated with lower emotional problems in girls from low risk family backgrounds, but not among boys or children from high risk family backgrounds (Cote et al., 2009). In addition, hostile parenting predicts a child's membership in a high physical aggression-high indirect aggression trajectory (Cote et al., 2007).

Peer victimization is unrelated to a child's genetic disposition towards aggression (Brendgen et al., 2008). Low peer acceptance places a child at a higher risk for victimization, while low social status is also an outcome of victimization (Card & Hodges, 2008). Among preschool children, teacher-rated relational aggression is associated with maturity in girls, while coercive and prosocial strategies of resource control, although aggressive, are also associated with maturity among boys and girls (Hawley, 2003). In addition, children employing these strategies of resource control are rated as the preferred playmates of their peers (Hawley, 2003).

Cultural differences in aggression have also been studied. For example, we know that Japanese undergraduate students report higher levels of physical aggression than Spanish undergraduate students, who report higher levels of verbal aggression, hostility, and anger. Across the two groups, boys score higher than girls on dimensions of physical aggression, verbal aggression, and hostility. (Ramirez, Andreu, & Fujihara, 2001)

Language is another variable thought to influence an individual's level of aggression. Specifically, there exists a negative correlation between expressive vocabulary and physical aggression, not accounted for by shared etiologies (Dionne, Tremblay, Boivin, Laplante, & Perusse, 2003). In studies of language development, it is important to control for a child's monolingual or bilingual status because we know that bilingual children experience an initial delay in speech compared to their monolingual peers, which may place them at higher risk for engaging in aggressive behaviors early in life.

*1.3.5 Hormones and aggression*

A strong link has been demonstrated between testosterone levels and aggression in rodents (Breuer, McGinnis, Lumia, & Possidente, 2001; Farrell & McGinnis, 2003). However, there have been contradictory findings regarding the existence of this link in primates (Dixson, 1980; Eberhart, Keverne, & Meller, 1980) and humans (Albert, Walsh, & Jonik, 1993; Harris, 1999; Mazur & Booth, 1998). Many studies investigating the association between testosterone and aggression in humans have used samples consisting of post pubertal boys (Bokhoven et al., 2006). However, exploring this association in early childhood is imperative because this is when testosterone levels are most stable.

Few studies have examined the association between testosterone and aggression early in life. From these studies we know that humans prenatally exposed to synthetic progestins show higher levels of physical aggression than their unexposed siblings (Reinisch, 1981). We also know that females with CAH, which are born with abnormally high levels of testosterone, score higher on aggression than females without CAH (Berenbaum & Resnick, 1997; Pasterski et al., 2007).

Cortisol is believed to be a moderating link between aggression and testosterone (Banks & Dabbs, 1996). In fact, many studies have found children with lower levels of cortisol to have higher levels of aggression (McBurnett, Lahey, Rathouz, & Loeber, 2000). Girls with conduct disorder also have lower levels of cortisol, compared to girls without conduct disorder (Pajer, Gardner, Rubin, Perel, & Neal, 2001). In addition, functional asymmetry, which depends on the secretion of hormones such as cortisol, and aggression, especially physical aggression, are inversely related in boys (Manning & Wood, 1997). However, the link between cortisol and testosterone in relation to aggression remains controversial, with mixed findings (Scerbo & Kolko, 1994).

# 2. PURPOSE AND HYPOTHESES

## 2.1 Purpose

The primary objective of this research is to test the hypothesis that the transient activation of the HPG axis early in the first year of life influences the expression of sex-linked behaviors that emerge in early childhood, specifically aggressive behaviors (Reinisch, 1981). As considerable research has demonstrated that a life-long process of gender socialization contributes to sex differences in adult behavior (Bussey & Bandura, 1999; Martin, Ruble, & Szkrybalo, 2002), an examination of hormones and behavior in very early development will arguably provide a strong test of a hormonal hypothesis.

There are no studies examining the association between the increased production of hormones in the early postnatal life (3 months) and sex-linked personality traits in early childhood. This research addresses this gap in our knowledge and therefore increases our understanding of gender role behavior. The use of both objective and subjective data to examine hormone-behavior relations in toddlers makes this project creative and original.

Knowledge of the interaction between social and hormonal influences on early development of behavior will be important in understanding the consequences of atypical hormone levels associated with disease (e.g., hypogonadism) or hormone disruptors (e.g., phylates) during early postnatal life. Research on the early social and hormonal factors thought to contribute to sex differences is also relevant to understanding the development of childhood disorders associated with sex-biased prevalence rates (e.g., autism, conduct disorder).

### 2.2 Hypotheses

We expect to replicate previous findings showing sex differences in social behavior. Specifically, we expect boys to obtain higher levels of aggression and activity, and lower levels of developed verbal ability. We also hypothesize that higher testosterone levels in early infancy will predict higher levels of male-typical behavior in the second year of life (i.e., more aggressive behavior, higher activity levels, and less developed verbal abilities). In addition, we expect to provide new information on the role of hormone levels in behavior by showing that higher levels of testosterone in early infancy development contribute to increased male-typical personality and social behavior in early childhood.

# 3. MATERIALS AND METHODS

## 3.1 Participants

Participants were 54 children between 18 and 24 months (32 males and 22 females) and their parents recruited from birth announcements in the local newspaper and commercially produced lists for research on early sex differences in behavior. All parents gave signed, informed consent. The study was approved by the Institutional Review Board.

## 3.2 Measures

### *3.2.1 Hormone markers at 3-months*

Saliva (<15 ml) from each infant was collected by a sterile DeLee suction catheter. Saliva samples were immediately stored at -80° C. Frozen samples were shipped overnight in dry ice to Salimetrics (State College, Pennsylvania), where salivary levels of testosterone were measured in duplicate using enzyme immunoassays (assay sensitivity < 1 pg/ml).

The ratio of the lengths of the second and fourth digits (2D: 4D) was calculated by obtaining a digital photo scan of the infant's right hand. The distance in millimeters from the basal crease to the tip of the second and fourth fingers was measured with digital vernier calipers. Two independent judges coded finger-lengths for each hand copy with excellent inter-rater reliability (r > .90).

*3.2.2 Temperament at 18-months*

Toddler's temperament was measured using the Brief Infant Toddler Social-Emotional Assessment (BITSEA) (Briggs-Gowan & Carter, 2002). This screener is intended to identify social-emotional problems. The questionnaire contains 42 items, making up the problem and competence scales. BITSEA was designed for children ages 12-36 months and written at a $4^{th}$ to $6^{th}$ grade reading level. The questionnaire takes approximately 7 to 10 minutes to complete. BITSEA is available in English, Spanish, and Turkish.

BITSEA has been shown to have internal consistency ranging between .65 and .79. Inter-rater reliability between mother and father ranges between .61 and .68. Test-retest reliability ranges from .85 and .87. Predictive validity of the measure is .71. Sensitivity has been documented to fall between 80% and 99% and specificity between 80% and 89%. Although not significant ($p = .06$), sex differences in the problem scale have been reported, with boys obtaining higher problem scores than girls. Boys also obtained lower competence scores than girls in each age group ($p < .05$), except in the 12 to 17 month old age group. (Briggs-Gowan & Carter, 2002) BITSEA has also been shown to correlate with corresponding scales on other measures of social-emotional and behavioral problems (Briggs-Gowan & Carter, 2007).

*3.2.3 Sex-linked behavior at 18-months*

Trained observers coded aggression and verbal ability during videotaped play sessions using Observer XT. Measures of direct aggression and global aggression were measured. Direct aggression was measured by coding incidents when the child 1. hit, bit, or kicked his or her parent, 2. threw toys at his or her parent, and 3. became aggressive towards toys or objects in the environment (Shaw, Keenan, & Vondra, 1994). A qualitative measure of global aggression was based on a coder's ratings of the intensity, force, and duration of aggression. Global aggression ratings ranged from 1 = unaggressive, 2 = mildly aggressive, 3 = moderately aggressive, to 4 = severely aggressive (Shaw et al., 1994). Excellent inter-rater reliability was obtained for measures of direct aggression ($r = .94$) and global aggression ($r = 1$).

A child's verbal ability was defined as the frequency of single words (e.g., dog, book, car, baby), two word phrases (e.g., mom sit, call dad, blue block), and sentences composed of more than two words (e.g., the puppy is happy, where is mom, I like trucks). A fourth category measured the duration of a child's vocalization, defined as times when a child was being vocal, but no recognizable words were being said (e.g., lalalalalala, ah, weeeeeee). Special precautions were taken to avoid coding times when a child was breathing loudly, crying, laughing, clearing his or her throat, or burping as vocalizations. Inter-rater reliability was $r = .98$.

Activity level during play sessions were measured using actigraphy (Ambulatory Monitoring, Inc – Basic Model). Actigraphs are small devices, approximately the size of a wristwatch, that measure motor movement. Children and parents wore the actigraphs during both play sessions. Actigraphs have been used to provide a measure of individual differences in activity levels, in previous research on infants and toddlers (Campbell, Eaton, & McKeen, 2002; Saudino & Zapfe, 2008).  Past studies have shown actigraphs to be a reliable and valid method for assessing activity level (Puyau, Adolph, Vohra, & Butte, 2002; Wood, Kuntsi, Asherson, & Saudino, 2008).

### 3.3 Procedure

Consistent with methods from previous research on temperament and aggression in early development (Keenan & Shaw, 1994; Shaw et al., 1994), child-parent dyads (either mom and baby or dad and baby) were scheduled to participate in two play exercises. During the 1st 8 minute free-play exercise, parents were instructed to remain focused on filling out a questionnaire and try not to interact with their child. During the 2nd 8 minute play exercise parents were instructed to play with their child like they normally do at home. Consistent with methods from previous research on sex-linked play preferences (Hines & Kaufman, 1994; Pasterski et al., 2005), a total of nine toys (3 male-typical, 3 female-typical, 3 gender neutral) were available during the free play exercises (see Figure 1). The sessions were video-taped and later scored by two raters blind to the study hypotheses. Following completion of the study, parents were given $10 dollar reimbursement for their travel expenses and received a t-shirt for their child.

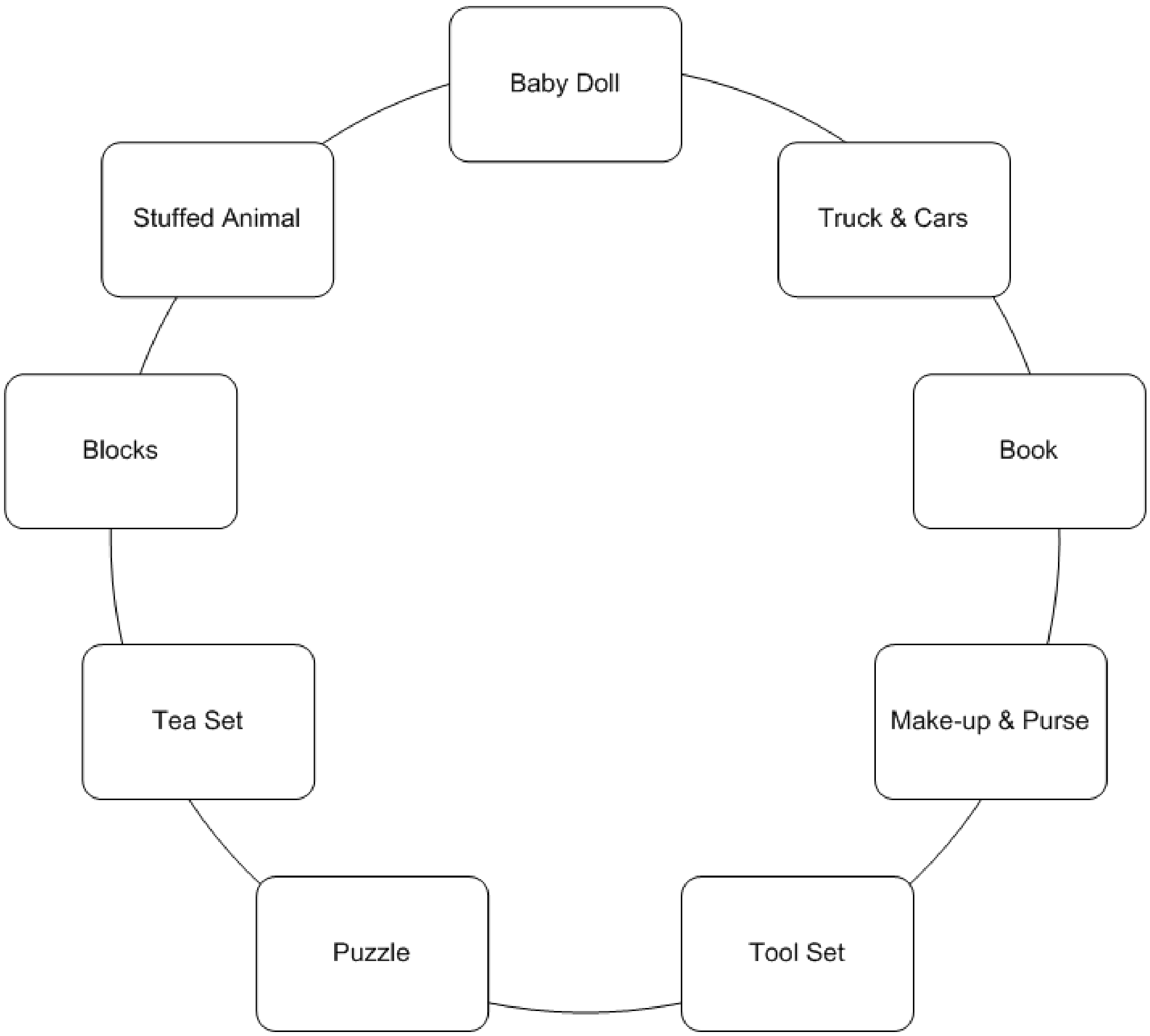


**Figure 1.** Toy configuration.

# 4. RESULTS

Preliminary data analyses were conducted to ensure all data were normally distributed and outliers were identified. One participant was excluded from additional analyses due to being a consistent outlier, both within and between genders, on boxplots for various aggression variables. Table 1 summarizes the sex differences.

## 4.1 Sex Differences

### *4.1.1 Hormone markers at 3-months*

Male infants compared to female infants had higher salivary testosterone levels (boys: 38.58 pg/ml ± 9.35 pg/ml *vs*. girls: 37.86 pg/ml ± 14.34 pg/ml, *d* = .06) and smaller digit ratios (boys: .920 mm ± .04 *vs.* girls: .938 mm ± .05, *d* = .40). However the sex differences in these measures were small and not statistically significant.

### *4.1.2 Temperament at 18-months*

Parents rated male and female infants similarly on the competence (boys: 17.74 ± 1.81 *vs.* girls: 17.86 ± 3.01, *d* = .05) and problem scales (boys: 9.52 ± 3.97 *vs.* girls: 8.59 ± 4.49, *d* = .22). Male and female infants were also rated similarly on internalizing problems (boys: 1.74 ± 1.37 *vs.* girls: 1.68 ± 1.39, *d* = .04), dysregulation problems (boys: 2.74 ± 1.73 *vs.* girls: 2.95 ± 1.89, *d* = .12), and autism spectrum disorders (boys: 3.71 ± 1.49 *vs.* girls: 3.64 ± 2.75, *d* = .03). However, males received significantly higher scores on the externalizing problems scale than females (boys: 2.42 ± 1.50 *vs.* girls: 1.55 ± 1.41, *d* = .60), $F\ (1, 51) = 4.60$, $p < .05$, partial $\eta^2 = .08$.

**Table 1.**

Sex Differences across Study Variables for Both Sessions Combined

| Variable | Males | Females | P | d |
|---|---|---|---|---|
| Hormones | | | | |
| Salivary T levels | 38.58 (9.35) | 37.86 (14.34) | .86 | .06 |
| 2D:4D ratios | .92 (.04) | .94 (.05) | .12 | .40 |
| BITSEA | | | | |
| Competence scale | 17.74 (1.82) | 17.86 (3.01) | .86 | .05 |
| Problem scale | 9.52 (3.97) | 8.59 (4.49) | .43 | .22 |
| Externalizing | 2.42 (1.50) | 1.55 (1.41) | .04* | .60 |
| Internalizing | 1.74 (1.37) | 1.68 (1.39) | .89 | .04 |
| Dysregulation | 2.74 (1.73) | 2.95 (1.89) | .67 | .12 |
| ASD | 3.71 (1.49) | 3.64 (2.75) | .90 | .03 |
| Aggression | | | | |
| Aggression at toys | 21.10 (17.96) | 15.05 (14.57) | .20 | .37 |
| Throw toys at parent | .10 (40) | .09 (.29) | .95 | .03 |
| Hit/kick/push parent | .39 (1.52) | .91 (2.47) | .35 | .25 |
| Total aggression | 21.58 (18.26) | 16.05 (14.39) | .24 | .34 |
| Global aggression | 2.26 (.86) | 1.82 (.66) | .05* | .57 |
| Verbal ability | | | | |
| 1 word | 39.58 (51.13) | 33.28 (25.08) | .60 | .16 |
| 2 word phrases | 6.00 (9.94) | 6.14 (9.87) | .96 | .02 |
| 2+ word sentences | .77 (3.25) | .64 (1.33) | .85 | .05 |
| Total words | 54.03 (65.58) | 47.18 (42.07) | .67 | .12 |
| Number of V | 54.39 (24.80) | 46.77 (24.00) | .27 | .31 |
| Duration of V | 47.94 (42.17) | 42.32 (35.21) | .61 | .14 |
| Activity level | 9499.73 (3401.98) | 8579.16 (3506.02) | .37 | .27 |

Note. T = Testosterone. ASD = Autism Spectrum Disorders. V = Vocalization.

* $p < .05$. ** $p < .01$.

*4.1.3 Aggression at 18-months*

ANOVA for repeated measures with sex (male, female) as a grouping factor and aggression type (aggression towards toys or objects in the environment, throwing toys at a parent, hitting/kicking a parent) as a repeated factor found no significant sex differences, $F(1, 51) = 1.40$, $p > .05$, *ns*, and no sex by aggression type interaction, Greenhouse-Geisser adjusted $F(1.02, 52.05) = 1.83$, $p > .05$, *ns*, such that male and female infants displayed similar levels of aggression towards toys or objects (boys: $21.10 \pm 17.96$ *vs.* girls: $15.05 \pm 14.57$, $d = .37$), throwing toys at their parents (boys: $.10 \pm .40$ *vs.* girls: $.09 \pm .29$, $d = .03$), and hitting/kicking their parents (boys: $.39 \pm 1.52$ *vs.* girls $.91 \pm 2.47$, $d = .25$).

The results also showed a significant main effect for aggression type, Greenhouse-Geisser adjusted $F(1.02, 52.05) = 57.44$, $p < .05$, partial $\eta^2 = .53$. Dependent samples t tests were conducted to assess which of the aggression types differed from one another, with each test conducted at an alpha level of .016. The results indicated that aggression directed at toys or objects in the environment ($M = 18.58$, $SD = 16.76$) was employed at significantly higher rates than throwing toys at their parents ($M = .09$, $SD = .35$), $t(52) = 8.02$, $p < .016$, and hitting/kicking their parents ($M = .60$, $SD = 1.96$), $t(52) = 7.75$, $p < .016$. Levels of throwing toys at their parent and hitting/kicking their parent were not significantly different, $t(52) = -.90$, $p > .016$, *ns*. The three types of aggression were added for each session and referred to as the infant's total level of aggression.

As expected, boys compared to girls generally received higher ratings of global aggression (boys: $2.26 \pm .86$ *vs.* girls: $1.82 \pm .67$, $d = .57$), $F(1, 52) = 4.07$, $p <.05$, partial $\eta^2 = .07$, and total aggression across the two play sessions (boys: 21.58 ± 18.26 *vs.* girls: 16.05 ± 14.40, $d = .34$), $F(1, 52) = 1.40$, $p > .05$, *ns*, although differences in the latter were small and non significant. ANOVA for repeated measures with sex (male, female) as the between subjects factor and play session (play session alone, play session with parent) as the within subjects factor was conducted to examine if total aggression scores varied for each play session by sex. The results showed no significant main effects for sex, $F(1,51) = 1.40$, $p > .05$, *ns*, or play session, $F(1,51) = 1.02$, $p > .05$, *ns*. Additionally, no sex by play session interaction was found, $F(1,51) = 1.20$, $p > .05$, *ns*. However, although not statistically significant, a trend emerged indicating that while boys and girls displaysed similar levels of aggression when playing alone (boys: 10.68 ± 13.48 *vs.* girls: 10.68 ± 11.92, $d = 0$), girls' level of aggression diminished while playing with their parents (10.68 ± 11.92 *vs.* 5.36 ± 5.51, $d = .57$), while boys maintained a consistent level of aggression accross both play sessions (10.68 ± 13.48 *vs.* 10.98 ± 14.71, $d = -.02$) (see Figure 2).

*4.1.4 Verbal ability at 18-months*

Male and female infants said similar numbers of single words (boys: 39.58 ± 51.13 *vs.* girls: 33.27 ± 25.08, $d = .16$), two words phrases (boys: 6.00 ± 9.94 *vs.* girls: 6.14 ± 9.87, $d = .02$), and sentences containing more than two words (boys: .77 ± 3.25 *vs.* girls: .64 ± 1.33, $d = .05$). Next, toddlers were divided into two verbal groups (children who could speak two word phrases, children who could not speak two word phrases) and a chi-square test of independence was conducted to see if girls were more likely to be in the group that could speak two word phrases (i.e., the advanced group). There was no

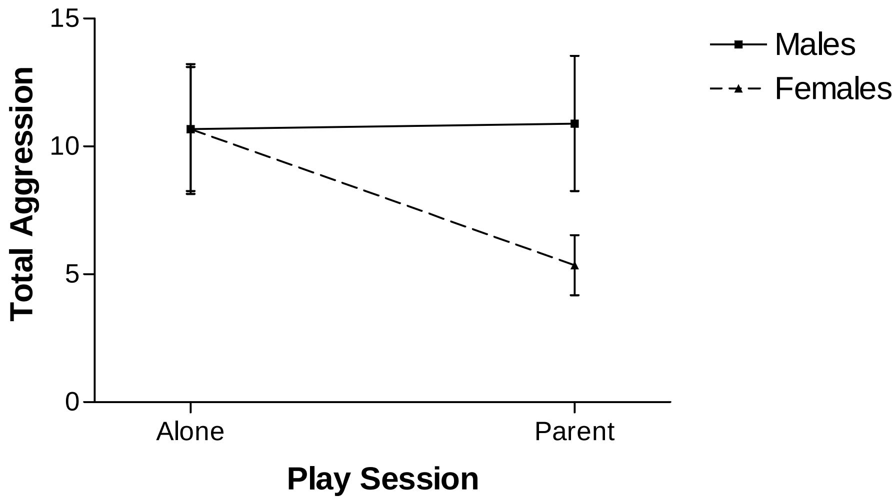


**Figure 2.** Infants' mean, total aggression scores across the two play sessions. No statistically significant relationships were obtained.

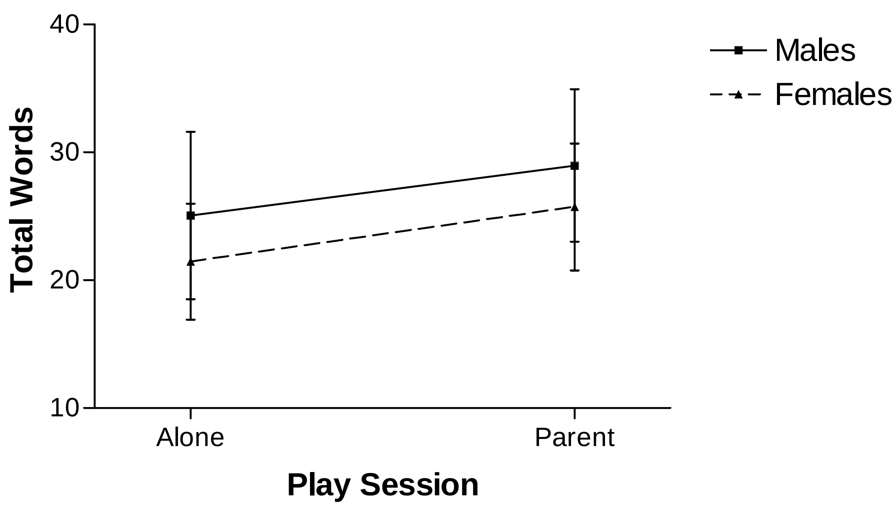


**Figure 3.** Infants' mean, total words across the two play sessions. No statistically significant relationships were obtained.

significant relationship between sex and verbal group, $\chi^2$ (1, N = 54) = 1.31, *ns*. Since no significant sex differences were observed within the different categories, we added across categories to identify the total words spoken by an infant, resulting in three indicators of vocal ability: total words spoken, total number of vocalizations, and total duration of vocalizations.

ANOVA for repeated measures with sex (male, female) as a grouping factor and total number of words spoken by infants during each session (total words during alone play session, total words during parent play session) as a repeated factor found no significant main effects for sex, $F(1, 51) = .19$, *ns*, or session, $F(1, 51) = 2.05$, *ns*, and no significant sex by session interaction, $F(1, 51) = .00$, *ns*. Indicating that male and female infants spoke roughly the same number of words while playing alone (boys: 25.06 ± 36.50 *vs.* girls: 21.45 ± 21.30, *d* = .12), and while playing with their parents (boys: 28.97 ± 33.19 *vs.* girls: 25.73 ± 21.30, *d* = .12) (see Figure 3). The overall consistency across sessions implies that this is a good estimate of their overall vocabulary size.

ANOVA for repeated measures with sex (male, female) as a grouping factor and total number of vocalizations during each session (total vocalizations during alone play session, total vocalizations during parent play session) as a repeated factor found a significant main effects for session, $F(1, 51) = 17.97$, $p < .05$, partial $\eta^2 = .26$, and a significant sex by session interaction, $F(1, 51) = 6.90$, $p = < .05$, partial $\eta^2 = .12$, such that children vocalized more when playing with their parent (boys: 34.26 ± 16.84 *vs.* girls: 25.05 ± 12.74, *d* = .62), than when playing alone (boys: 20.13 ± 11.67 *vs.* girls:

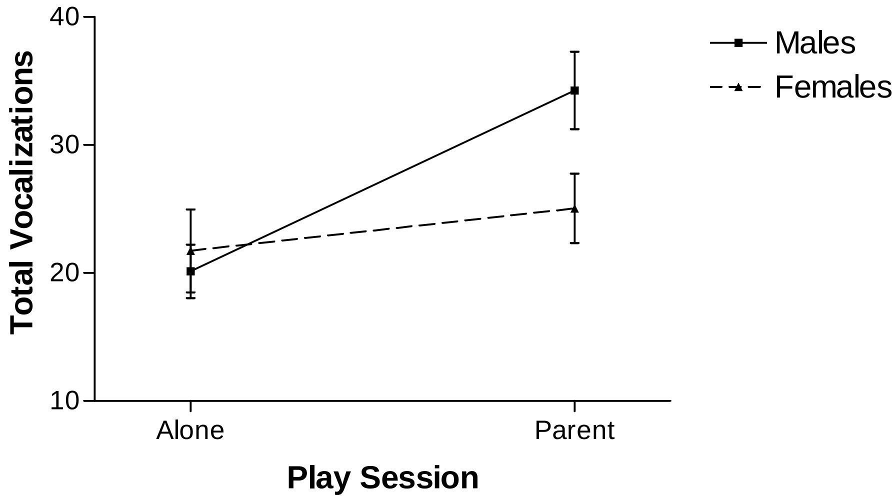


**Figure 4.** Infants' mean, total number of vocalizations across the two play sessions. A significant main effect for session and sex by session interaction were obtained, such that infants, especially males, vocalized more when playing with their parent than when playing alone.

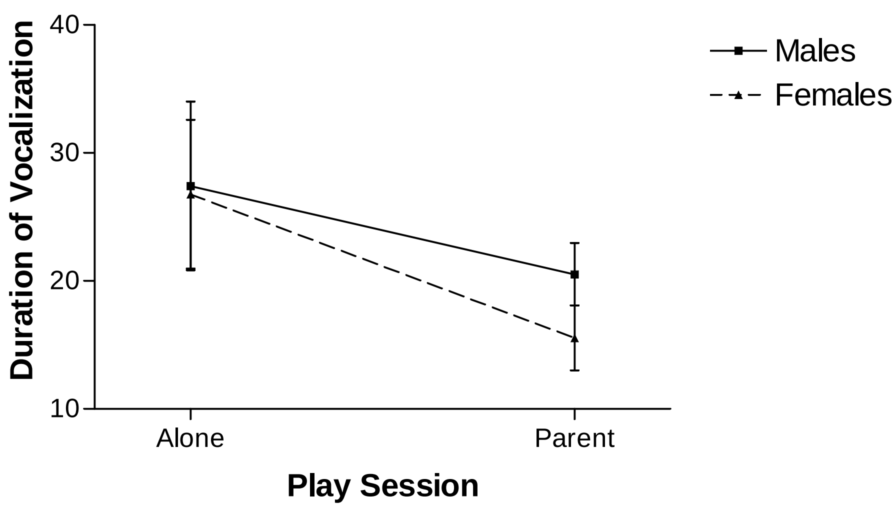


**Figure 5.** Infants' mean, total duration of vocalization across the two play sessions. A significant main effect for session was obtained, such that infants' total duration of vocalization was larger when infants played alone than when playing with their parents.

21.73 ± 15.73, *d* = .12) (see Figure 4). This increase in vocalization is substantially larger for male infants. No significant main effect for sex was found, $F\ (1, 51) = 1.25$, $p > .05$, *ns*.

Lastly, ANOVA for repeated measures with sex (male, female) as a grouping factor and total duration of vocalizations during each session (total duration of vocalizations during alone play session, total duration of vocalizations during parent play session) as a repeated factor found a significant main effect for session, $F\ (1, 51) = 4.36$, $p < .05$, partial $\eta^2 = .08$, such that the total duration of vocalizations was greater when children played alone (boys: 27.42 ± 36.65 *vs.* girls: 26.77 ± 27.27), than when children played with their parent (boys: 20.52 ± 13.58 *vs.* girls: 15.55 ± 11.93) (see Figure 5). No significant main effect for sex, $F\ (1, 51) = .26$, $p > .05$, *ns*, or sex by session interaction, $F\ (1, 51) = .62$, $p > .05$, *ns*, was found.

*4.1.5 Activity level at 18-months*

ANOVA for repeated measures with sex (male, female) as a grouping factor and infant's activity level during each session (activity level during alone play session, activity level during parent play session) as a repeated factor found no significant sex differences, $F\ (1, 47) = .83$, $p > .05$, *ns*, and no sex by play session interaction, $F\ (1, 47) = .47$, $p > .05$, *ns*. However, a main effect for session was obtained, $F\ (1, 47) = 38.68$ $p < .05$, partial $\eta^2 = .45$, such that male and female infants were more active while playing alone (boys: 5921.43 ± 2096.35 *vs.* girls: 5228.63 ± 1911.81) than while playing with their parent (boys: 3578.30 ± 2122.63 *vs.* girls: 3350.53 ± 2114.46) (see Figure 6).

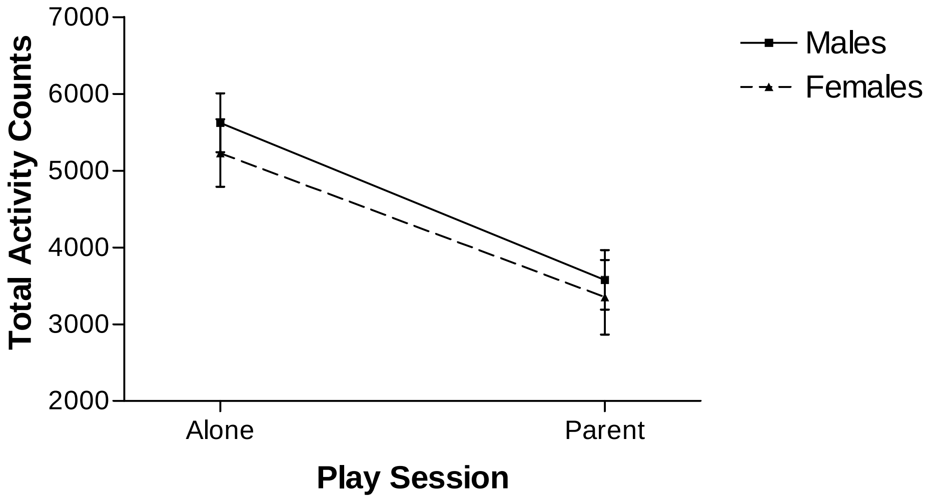


**Figure 6.** Infants' mean, total activity counts across the two play sessions. A significant main effect for session was obtained, such that infants were more active while playing alone than when playing with their parent.

**Table 2.**
Correlations between Hormones and Behaviors

| | Hormones at 3-months | | Behaviors at 18-months | | | | |
|---|---|---|---|---|---|---|---|
| Variable | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| 1. Salivary T levels | — | -.16 | .16 | -.37 | -.32 | -.51* | -.06 |
| 2. 2D:4D ratios | .42 | — | -.33 | -.31 | .46* | -.09 | -.32 |
| 3. Total aggression | -.28 | -.43 | — | .44* | .21 | -.10 | .32 |
| 4. Global aggression | -.23 | -.51* | .56** | — | -.08 | .14 | .29 |
| 5. Total words | -.02 | .26 | -.20 | .05 | — | .02 | -.08 |
| 6. Total vocal duration | -.44 | -.05 | .18 | .30 | .48* | — | .39* |
| 7. Total activity | -.03 | -.55 | .09 | .39 | .32 | .05 | — |

Note. T = Testosterone. Male and female scores are represented above and below the diagonal, respectively.
* p < .05. ** p < .01.

In addition, activity levels across both sessions were added to determine a child's total activity. Consistent with our hypothesis, total activity counts were generally higher in boys compared to girls (boys: 9499.73 ± 3401.98 *vs.* girls: 8579.16 ± 3506.02, $d$ = .27). However this sex difference was small and not statistically significant.

**4.2 Hormones and Behavior**

Table 2 summarizes the correlations between hormones and behavior. Hierarchical regression analyses were conducted using measures of sex-linked behaviors at 18-months as dependent variables (total aggression, total words spoken, total duration of vocalization, total activity level). For each model, sex, age, and 3-month digit ratios were entered in the first step and 3-month salivary testosterone levels were entered in the second step.

In the first analysis, a significant relationship between vocal duration and testosterone was obtained. Such that, lower salivary testosterone levels (i.e., more female-typical) predicted longer durations of vocalization. In the first block, sex, age and digit ratios accounted for a small, non-significant portion of the variance in total duration of vocalization, $\Delta R^2 = .05$, F (3, 30) = .52, *ns*. The addition of salivary testosterone produced a significant effect, $\Delta R^2 = .20$, F (1, 29) = 7.87, $p < .05$. Examination of individual beta weights indicated that salivary testosterone, $\beta = -.47$, $p < .05$, contributed significantly to the variance in the group.

Additionally, significant relationships between age and total words, and digit ratios and total words were also present. In the first block, age and digit ratios accounted for a significant portion of the variance in the number of words they said, $\Delta R^2 = .40$, $F(3, 30) = 6.65$, $p < .05$. The addition of salivary testosterone produced small effects, $\Delta R^2 = .02$, $F(1, 29) = 0.87$, *ns*. Examination of individual beta weights indicated that the infant's age, $\beta = .45$, $p < .05$, contributed significantly to the variance in the group, such that older infants spoke more words. Moreover, the infant's digit ratios, $\beta = .38$, $p < .05$, also contributed significantly to the variance in the group, such that infants with bigger digit ratios (i.e., more female-typical) spoke more words.

Furthermore, the regression for activity level within sex was not significant for either the first model containing sex, age, and digit ratios, $\Delta R^2 = .24$, $F(3, 27) = 2.81$, $p = .06$, or the second model containing testosterone, $\Delta R^2 = .00$, $F(1, 26) = 0.02$, *ns*. However, the individual beta weights indicated that the infant's digit ratios, $\beta = -.44$, $p < .05$, contributed significantly to the variance in the group, such that infants with smaller digit ratios (i.e., more male-typical) were more active.

Lastly, the regression for total aggression within sex was not significant for either the first model containing age and digit ratios, $\Delta R^2 = .17$, $F(3, 30) = 2.01$, *ns*, or the second model containing testosterone, $\Delta R^2 = .00$, $F(1, 29) = 0.02$, *ns*. However, the individual beta weights indicated that the infant's digit ratios, $\beta = -.42$, $p < .05$, contributed significantly to the variance in the group, such that infants with smaller digit ratios (i.e., more male-typical) were more aggressive.

To verify that these results were not obtained solely as a consequence of a sex difference, separate hierarchical regression analyses were conducted for each sex, using the measures of sex-linked behaviors that reached significance when males and females were combined as dependent variables (total words spoken, total duration of vocalizations). For each model, age and 3-month digit ratios were entered in the first step and 3-month salivary testosterone levels were entered in the second step. Results of these analyses for female infants showed that the variance in the two sex-linked behavior measures could not be significantly accounted for by any of the models or predictor variables.

However, for male infants, lower salivary testosterone levels (i.e., more female-typical) predicted longer duration of vocalization. In the first step, age and digit ratios accounted for a small, non-significant portion of the variance in total duration of vocalization, $\Delta R^2 = .03$, $F(2, 18) = .32$, *ns*. The addition of salivary testosterone produced a significant effect, $\Delta R^2 = .30$, $F(1, 17) = 7.74$, $p < .05$. Examination of individual beta weights indicated that salivary testosterone, $\beta = -.56$, $p < .05$, contributed significantly to the variance in the group.

In addition, for male infants, being older predicted saying more words. In the first step, age and digit ratios accounted for a significant portion of the variance in the number of words they said, $\Delta R^2 = .44$, $F(2, 18) = 7.04$, $p < .05$. The addition of salivary testosterone produced small effects, $\Delta R^2 = .05$, $F(1, 17) = 1.60$, *ns*. Examination of individual beta weights indicated that the infant's age, $\beta = .50$, $p < .05$, contributed significantly to the variance in the group. Table 3 summarizes the regression coefficients within sex, while Table 4 and Table 5 summarize the regression coefficients for males and females, respectively.

**Table 3.**

Hierarchical Regression Models for All Infants

| Trait | Coefficient | t | P |
|---|---|---|---|
| Total Aggression | | | |
| 1. Sex | .07 | .41 | .69 |
| 1. Age | .02 | .10 | .92 |
| 1. 2D:4D at 3-months | -.42 | -2.41 | .02 |
| 2. Salivary T at 3-months | .03 | .15 | .88 |
| Total Words | | | |
| 1. Sex | -.22 | -1.47 | .15 |
| 1. Age | .45 | 3.05 | .01 |
| 1. 2D:4D at 3-months | .38 | 2.61 | .01 |
| 2. Salivary T at 3-months | -.14 | -.93 | .36 |
| Duration of Vocalization | | | |
| 1. Sex | -.19 | -1.14 | .27 |
| 1. Age | -.07 | -.39 | .70 |
| 1. 2D:4D at 3-months | -.06 | -.34 | .73 |
| 2. Salivary T at 3-months | -.47 | -2.81 | .01 |
| Total Activity | | | |
| 1. Sex | -.11 | -.61 | .55 |
| 1. Age | -.04 | -.24 | .81 |
| 1. 2D:4D at 3-months | -.44 | -2.48 | .02 |
| 2. Salivary T at 3-months | -.02 | -.14 | .89 |

Note. T = Testosterone. 1. = Model One. 2. = Model Two.

**Table 4.**
Hierarchical Regression Models for Males

| Trait | Coefficient | t | P |
|---|---|---|---|
| Total Words | | | |
| 1. Age | .49 | 2.72 | .01 |
| 1. 2D:4D at 3-months | .28 | 1.52 | .15 |
| 2. Salivary T at 3-months | -.22 | -1.26 | .22 |
| Duration of Vocalization | | | |
| 1. Age | -.16 | -.79 | .44 |
| 1. 2D:4D at 3-months | -.19 | -.89 | .38 |
| 2. Salivary T at 3-months | -.56 | -2.78 | .01 |

Note. T = Testosterone. 1. = Model One. 2. = Model Two.

**Table 5.**
Hierarchical Regression Models for Females

| Trait | Coefficient | t | P |
|---|---|---|---|
| Total Words | | | |
| 1. Age | .34 | 1.03 | .33 |
| 1. 2D:4D at 3-months | .46 | 1.35 | .21 |
| 2. Salivary T at 3-months | -.04 | -.13 | .90 |
| Duration of Vocalization | | | |
| 1. Age | .40 | 1.33 | .22 |
| 1. 2D:4D at 3-months | .21 | .66 | .52 |
| 2. Salivary T at 3-months | -.37 | -1.20 | .26 |

Note. T = Testosterone. 1. = Model One. 2. = Model Two.

# 5. DISCUSSION AND CONCLUSION

## 5.1 Discussion

The primary objective of this research was to test the hypothesis that the transient activation of the HPG axis early in the first year of life influences the expression of sex-linked behaviors that emerge in early childhood. Support for this hypothesis comes from our results which demonstrate that hormone markers associated with higher (i.e., more male-typical) testosterone were related to more aggressive behaviors, higher activity levels, expression of fewer total words, and a shorter duration of time spent vocalizing. This study replicates previous findings showing that sex differences in levels of prenatal and early postnatal life testosterone were associated with the expression of some aspects of gender linked behavior (Friederici et al., 2008; Hines & Kaufman, 1994; Pasterski et al., 2007). Therefore, in contrast to findings in other nonhuman primates (Nevison, Brown, & Dixson, 1997), the present research suggests that hormone levels in early postnatal life may contribute to the development of gender phenotypes.

Behaviors that in older children vary between boys and girls were assessed in infants at 18 months of age, during a play session with and without their parent or caretaker. Sex-linked behaviors coded in the two play sessions included aggression, activity levels, and language ability. Overall, boys were more aggressive, engaged in higher levels or activity, and showed less developed language ability. However, these expected sex differences were small and did not reach statistical significance.

Although no significant sex differences were obtained for total aggression (d = .34), our effect size is consistent with those reported in observational studies of direct aggression in children (d = .37) (Card et al., 2008). Unlike total aggression, we did obtain a significant sex difference for global aggression, such that boys received significantly higher ratings than girls. Perhaps, global aggression is a better measure of aggressive behavior because in addition to capturing the frequency of aggressive behavior, like our total aggression scale, it also captures additional aspects of aggression, such as intensity, duration, and force. The social expectations of our coders, based on gender stereotypes, could have also influenced their decision to rate boys as being more aggressive than girls.

Overall, our sample utilized low levels of aggressive behavior. Moreover, both boys and girls were more likely to direct aggression towards toys or objects in the environment than at their parent. Even though we only measured physical forms of aggression, one can argue that there is a qualitative difference between hitting a person and hitting an object, with the former recognized as less severe and more acceptable than the latter. It appears that children make this distinction before the age of two.

We also found a trend indicating that boys and girls displayed similar levels of aggression when playing alone. However, girls' level of aggression diminished while playing with their parents, while boys maintained a consistent level of aggression accross both play sessions. Together, these findings suggest that parents tolerate more aggressive behaviors in boys than in girls.  If so, then this differential response to aggressive behavior in early infancy may contribute to the larger gender differences in aggressive behavior observed in older children (Pasterski et al., 2007).

Consistent with previous studies of sex differences in activity levels of 2 year olds (d = .24-.48), males obtained higher activity levels than females (d = .27) (Saudino, 2009; Saudino & Zapfe, 2008). Perhaps this sex difference reflects the actigraphy's greater sensitivity to gross motor movements, which may be more characteristic of male-typical play, and less sensitivity to fine motor movements, which may be more characteristic of female-typical play. Additionally, male and female infants were more active while playing alone than while playing with their parent. These findings suggest that parents may help their children focus their attention and energy on one activity or task, thereby decreasing overall activity levels.

Contrary to past studies (Lutchmaya, Baron-Cohen, & Raggatt, 2002; Reilly et al., 2009), no sex differences were found in the utilization of single words, two word phrases, or sentences longer than two words. When categorized into two groups (those that could speak two word phrases and those that could not speak two word phrases), females were slightly more likely than males to belong to the group that could speak two word phrases (i.e., the advanced group). These results could reflect a tendency for boys to engage in more repetitive behaviors, including the repetition of identical words or vocalizations, delaying their ability to form more complex sentence structures.

Furthermore, male and female infants spoke roughly the same number of words while playing alone and while playing with their parent. The overall consistency across the two sessions implies that this is representative of the infants' vocabulary size. In addition, children's number of vocalizations increased, while their total duration of vocalization decreased when they played with their parent. One reason why infants may have obtained longer durations of vocalization while playing alone is that they could have experienced an extinction burst. Children as young as 5-months old understand that their vocalizations are met by responses from caregivers (Goldstein, Bornstein, & Schwade, 2009), suggesting that our sample of 18-month olds also understood that their vocalizations usually result in caregiver attention. Because parents were instructed to minimize interactions with their infants, it is possible that when vocalizing was not followed by parental attention, children vocalizations increased. It could also be that children vocalized for shorter durations while playing with their parent because they were more engaged in reciprocal conversations.

As mentioned earlier, the primary objective of this study was to test whether higher testosterone levels in prenatal and early postnatal life would predict higher levels of male-typical behavior in the second year of life (i.e., higher aggressive behavior, higher activity levels, and lower verbal ability). As expected, infants with smaller digit ratios (i.e., more male-typical) were more aggressive, more active, and spoke less words. Contrary to our general hypothesis, no significant relationship between early postnatal testosterone and aggressive behaviors, activity levels, or total words was obtained. However, a significant relationship between early postnatal testosterone and duration of vocalization was obtained, such that lower salivary testosterone levels (i.e., more female-typical) predicted longer durations of vocalization with both sexes together, and within males only.

Similarly, past research has shown that foetal testosterone is predictive of vocabulary size in infants at 18- and 24-months of age (Lutchmaya et al., 2002). However, this finding was only obtained with both sexes together, but not within each sex. Key differences between this study and ours include their use of prenatal testosterone which differs from our use of early postnatal testosterone, and their use of the Communicative Development Inventory (CDI), a parental report measure of vocabulary size which differs from our use of behavioral coding to obtain measures of vocal ability. Together these findings and our results based on behavioral observations of children's vocalizations, suggest that prenatal and early postnatal testosterone levels affect language development.

Additional support for the role of testosterone in the language organization of infants comes from a study that had 4-week old infants undergo a phoneme discrimination task, while obtaining Electroencephalogram (EEG) recordings. The results showed that females displayed a phonological discrimination effect that was bilaterally distributed. While, males with high testosterone (i.e., more male-typical) levels showed no discrimination effect and those with low testosterone (i.e., more female-typical) levels showed a discrimination effect that was left-lateralized. Generally, it is believed that females' less lateralization enables them to use both hemispheres of their brain which contributes to their superiority over males in most verbal tasks. (Friederici et al., 2008)

If early postnatal testosterone levels do in fact contribute to the organization of language in the infant brain, it is possible that higher levels of testosterone could lead to developmental delays in language formation, placing a child at risk for developing deficits in language impairments as early as 4-weeks of age. Further, if the postnatal testosterone surge experienced in early infancy is another critical period for the organization of language and other gender-linked behaviors, then exposure to hormone disruptors at this time may alter the development of these behaviors. Further, if the postnatal testosterone surge experienced in early infancy is another critical period for the organization of language and other gender-linked behaviors, then exposure to hormone disruptors at this time may alter the development of these behaviors. Such disruptors include chemicals, such as bisphenol A (BPA), which is found in most plastics, including baby bottles. In short, our findings suggest that environmental toxins may impact normal child development.

Although our definition of vocal abilities allowed for a richer measure of this multi-faceted variable, our idiosyncratic definition was also a limitation of our study preventing us from determining whether more single words simply meant more repetition of single words or was truly representative of a larger vocabulary size. Vocabulary size is traditionally used as a standard indicator of language ability in infants. Our study was also subject to human error and biases which could have occurred during the behavioral coding and data entry phases. In addition, our small sample size may have prevented us from reaching statistically significant relationships among variables. Alternatively, our small sample size may also lead some to question the reliability of our results. Therefore, it will be important to replicate these findings in research using a larger number of children.

## 5.2 Conclusion

In conclusion, we found that boys were more aggressive, engaged in higher levels or activity, and showed less developed language ability. In addition, our results demonstrate that hormone markers associated with higher (i.e., more male-typical) testosterone were related to more aggressive behaviors, higher activity levels, expression of fewer total words, and a shorter duration of time spent vocalizing. A novel finding was that higher testosterone (i.e., more male-typical) levels in early postnatal life predicted less time spent vocalizing, for both sexes together and within males. The present research suggests that hormone levels in early postnatal life may contribute to the development of gender phenotypes, potentially making this a critical period for the development of language and other gender-linked behaviors.

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